Comparison of Direct and Indirect Sampling Methods for Tarnished Plant Bug (Hemiptera: Miridae) in Flowering Cotton

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ABSTRACT A complex of hemipterans, especially the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), has become a major target of insecticides in flowering cotton, *Gossypium hirsutum* L., in the mid-southern United States. Sampling protocols for this complex during this period of cotton development are poorly established, resulting in uncertainty about when infestations warrant treatment. Nine direct and indirect sampling methods were evaluated for bias, precision, and efficiency in cotton throughout the Mid-South during 2005 and 2006. The tarnished plant bug represented 94% of the bug complex in both years. Sweep-net and black drop-cloth methods were more efficient than other direct sampling methods, but they were biased toward adults and nymphs, respectively. Sampling dirty blooms was the most efficient indirect sampling method. The sweep-net, whole-plant, and dirty-bloom methods were more accurate than the other sampling methods evaluated based on correlations with other sampling methods. Variability attributed to the person collecting the sample was significant for all sampling methods, but least significant for the dirty-square method. Further research is needed to establish thresholds based on sweep-net, drop-cloth, dirty-square, and dirty-bloom sampling methods as these methods provide the best combinations of accuracy and efficiency for sampling tarnished plant bugs in cotton.

KEY WORDS *Lygus lineolaris*, accuracy, efficiency, bias

Several species of Hemiptera occur in cotton, *Gossypium hirsutum* L., in the mid-southern United States, namely, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), clouded plant bug, *Neurocolpus nubilus* (Say) (Hemiptera: Miridae), southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), green stink bug, *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae), and brown stink bug, *Euschistus servus* (Say) (Hemiptera: Pentatomidae). These species form an important pest complex of cotton in the Mid-South (Layton 2000), with tarnished plant bug frequently requiring insecticide applications before the cotton flowering period (Black 1973). Before 1995, infestations of the bug complex during the flowering period were often controlled by insecticides directed at other pests, so damage from bugs in flowering cotton was rare. However, since the near-eradication of the boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), and wide-scale adoption of transgenic *Bacillus thuringiensis* (Bt) cotton, foliar applications for most pests have been reduced. One consequence of this change is that hemipterans have become a prominent pest complex in flowering cotton. Control costs and crop losses associated with bugs have increased dramatically, with four to eight insecticide applications targeted at bugs in some years (Williams 2006).

The tarnished plant bug damages cotton in both adult and nymph stages by piercing the tissue and injecting salivary enzymes into the plant. Plant development is locally disrupted, causing abortion or malformation of the affected plant part (Layton 2000). Flower buds (squares) and small bolls are preferred feeding sites in cotton, so feeding leads to aborted squares, damaged anthers in the flower, sunken lesions on the outside of the boll, or stained lint and a wart-like growth on the inside of the boll.

Action thresholds have been developed for many pests to prevent economic losses in a particular field. To determine whether a threshold has been reached, an accurate pest density estimate must be made from a subsample of the population. Accuracy is a function of bias and precision (Binns et al. 2000), bias is a measure of how well the average sample density re-

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flects the actual field density, and precision is a measure of the similarity of samples drawn from a single population. In addition to these attributes, efficiency is needed to minimize the cost or effort of collecting the sample. A consistent, known bias or lack of bias, high precision, and high efficiency are desirable characteristics of a sampling method. Measures of precision and correlation to the actual pest density are most appropriate when the goal is to estimate the size of a population. However, when the goal is to classify a population as being above or below a predetermined criterion such as a spray threshold, the best test of a sampling method is to determine the probability of classifying the population correctly.

Considerable work has addressed efficient and accurate methods for sampling plant bugs and their damage during the preflowering stages of cotton development (Ellington et al. 1984, Fleischer et al. 1985). Consequently, agricultural pest managers have become comfortable with the sampling procedures and action thresholds for tarnished plant bugs in preflowering cotton where sweep-net sampling plus square-retention counts are used to determine the need for insecticide applications (e.g., Stewart and Lentz 2005). Unfortunately, there is no consensus on sampling methods for plant bugs during the flowering stages of cotton development. Pest managers have traditionally scouted flowering cotton by visually examining fruiting structures for other insect pests. Many continue to use this method, even though the target pests have changed. Thoroughness of visual searches for bugs in cotton varies considerably among individuals, with some examining a few squares and bolls per plant and others examining all parts of the plant. As a result, insect per plant thresholds based on a visual sampling method are reached at very different actual pest densities and depend more on the method and experience of the sampler than on scientific research.

Sweep nets and drop cloths are the two most common sampling methods evaluated by researchers for estimating *L. lineolaris* density. Most have found drop-cloth sampling to be more accurate than sweep-net sampling for estimating *L. lineolaris* infestations in flowering cotton (Young and Tugwell 1975, Snodgrass 1993). Consequently, flowering cotton treatment thresholds for *L. lineolaris* are often based on drop-cloth samples (e.g., Catchot 2005). Previous sampling research on both *L. lineolaris* and *L. hesperus* has tried to compare these more efficient relative sampling methods to absolute densities obtained by using a clamshell (Ellington et al. 1984) whole plant bag samples (Byerly et al. 1978, Garcia et al. 1982), visual search (Fleischer et al. 1985) or suction-type devices (Race 1960). Unfortunately, it becomes apparent from reading these papers that none of these sampling methods provide an absolute density estimate as these methods sometimes disagree with each other (Fleischer et al. 1985) or produce lower density estimates than relative sampling methods (Garcia et al. 1982). Young and Tugwell (1975) used small cages to estimate absolute nymph density, but adults could not be sampled using this method. Zink and Rosenheim (2004) used larger field cages to estimate absolute density of *L. hesperus* adults and nymphs, but the cage size and time required to make a single collection limited the number of samples that could be collected, making it impossible to compare the precision of their absolute method to relative sampling methods. Given this previous work in sampling cotton, no sampling method has been shown to reliably provide an absolute estimate of *Lygus* spp. density, so these sampling methods should be regarded as relative methods. As a result, sampling accuracy can only be estimated by comparing relative sampling methods.

In comparisons of drop-cloth and sweep-net methods, Gore (2005) found a relatively good correlation between these two methods, whereas Stewart et al. (2001) found that the relationship between these two methods varied, in part because drop cloths were better at detecting changes in immature densities, and sweep nets were better at detecting changes in adult densities. Drop cloths made with white fabric have traditionally been used for sampling in cotton. However, drop cloths made from black fabric have recently been adopted by some pest managers. Because thresholds are frequently based on the drop cloth, it is important to know whether the change of colors makes any difference in the number of insects observed. Although drop-cloth sampling seems to be preferred by researchers, some pest managers are reluctant to use drop cloths because of the perceived time and effort required for sampling.

Recent data suggest that plant-based monitoring procedures, such as numbers of damaged or frass-stained squares, may be more reliable than insect counts (Gore 2005). Plant-based boll injury thresholds have already been adopted across much of the cotton belt for stink bugs. These thresholds call for treatment when 10–20% of thumb- or quarter-sized bolls (≈1.5–2.0 cm in diameter) show internal evidence of injury such as warts on the carpel wall and lint staining. This approach was primarily validated in the southeast (Greene et al. 2001), where stink bug infestations in cotton are more common than tarnished plant bug infestations.

Variability among samplers and the influence of varying field conditions are other components of sampling that need to be considered when comparing sampling methods. Direct counts are known to vary among samplers (Morris 1960, Powell et al. 1996), whereas other sampling methods may provide more consistent data among samplers. Leaf wetness and wind speed have been reported to reduce efficiency of the sweep net (Cherry et al. 1977), but the impact of these factors on other sampling methods are unknown. To identify accurate and efficient sampling methods for *L. lineolaris* in flowering cotton, we evaluated numerous sampling methods throughout the Mid-South during 2005 and 2006.

Materials and Methods

Comparisons of insect-based and plant-based sampling methods (Table 1) for the bug complex were

Table 1. Description of the sampling methods evaluated

Method	Sample unit description
Insect-based methods	
Drop cloth	91- × 76-cm black cloth placed between two rows with the cotton from both sides vigorously shaken over the cloth (1.5 row-meter)
Sweep net	25 sweeps through the top of the canopy by using a 38-cm sweep net
Whole plant	Inspection of the terminal region (top two or three nodes), two large squares, one fresh bloom and one medium-sized boll on 25 plants
Squares	Inspection of 25 large flower buds (squares)
Blooms	Inspection of 25 fresh, white open flowers (blooms)
Plant-based method	
Dirty squares	Inspection of 25 large squares for external feeding signs (yellow stains)
Dirty blooms	Inspection of 25 fresh blooms for damaged anthers
External bolls	Inspection of 25 medium-sized bolls for sunken lesions on the carpel wall
Internal bolls	Internal inspection of 25 medium-sized bolls for wart-like growths on the carpel wall or stained lint in one or more locks

conducted in 2005 and 2006 on commercial cotton fields in Arkansas, Louisiana, Mississippi, and Tennessee. Row spacing ranged from 76 to 97 cm.

2005. Nine sampling methods (Table 1) were evaluated on 120 fields between 30 June and 6 September with cotton maturity ranging from early flower to zero nodes above white flower. Four sites were sampled in every field, and each site was typically sampled by a different individual using all nine methods. Five methods counted adult and immature bugs, whereas four methods sampled plant symptoms of bug feeding. Counts and sampling time were recorded for each method. Time of day, average plant height, average number of nodes above the first position white flower (NAWF), temperature, wind speed, and leaf wetness also were recorded in each field to enable an evaluation of the impact of these factors on each sampling method.

2006. As shown in the results, direct counts of bugs in squares and flowers showed little potential compared with the other methods in 2005, so these two sampling methods were omitted in 2006. Time of day was added as a controlled variable, so instead of sampling the field once per day as in 2005, 60 fields were sampled with the sweep-net, drop-cloth, and whole-plant methods during morning (7–9 a.m.), noon (11 a.m.–2 p.m.), and late afternoon (4–6 p.m.) of the same day. These 60 fields were sampled with all the indirect methods once per day as in 2005. Sampling took place from 6 July to 16 September, with cotton maturity ranging from early flower to zero nodes above white flower. As in 2005, four sites were sampled in each field by a different sampler. Samplers sampled a different site in the morning, noon, and later afternoon sampling periods, so variability within the field was not confounded with the sampler collecting the data. To address sampler influence on the data, records were kept on the person who collected each sample in 2006 to permit evaluation of the sensitivity of each sampling method to the sampler.

Black versus White Drop Cloth. Drop cloths (76 by 91 cm) of both colors were used by four samplers in 15 fields of flowering cotton containing *L. lineolaris* adults and all sizes of nymphs. Counts for each sampler from both drop-cloth colors were compared using a paired *t*-test.

Data Analyses. Counts collected for each method in each field on a single date were considered a sample (all sites in a field pooled). Experimental design was a randomized complete block with sampling methods as the treatments. Individual fields on a single date were the blocks. Analysis of direct sampling methods was done only for *L. lineolaris* as other bug species were not very abundant. However, because the damage of all the bug species is similar, plant-based damage estimates were compared with tarnished plant bug equivalents, where one clouded plant bug was set equal to 1.5 *L. lineolaris* and one stink bug was equal to three *L. lineolaris* based on thresholds for the different bug species currently used in the Mid-South (Catchot 2005, Stewart and Lentz 2005). To stabilize the variances, data were natural-log transformed and the transformed data were used in all analyses. Differences were regarded as significant at $\alpha = 0.05$.

To determine the most accurate relative sampling method without having a reliable absolute method we assumed that each relative sampling method used in this study equally reflected the actual *L. lineolaris* density and created a composite score for each field based on all the sampling methods. This composite score was the sum of counts for each method in each field after standardizing counts based on the overall mean and variance of that method to assure that all methods influenced the composite score equally. Although this approach is not an independent measure of density, it reflects the total knowledge of bug density for each field and is therefore expected to be a better approximation of the actual density than any single method. All methods were significantly correlated to each other (data not shown), so no method was contradicting any other method when evaluated over all samples. Correlations were then made between raw sampling method counts and the composite scores.

Similarly, rather than using a single method to determine whether or not the pest density exceeded a threshold, threshold classifications for individual methods were compared with the threshold classification of the majority of sampling methods. Some sampling methods have established thresholds in cotton, but other sampling methods do not. For this comparison we used the existing 1.6 *L. lineolaris* per row-meter drop-cloth threshold in Mississippi (Catchot 2005) and set all other thresholds at an equivalent level based on mean counts for each sampling method (e.g., sweep net equivalent = 1.6 × mean sweep-net density/mean drop-cloth density). This is not to imply that thresholds should be set at this level, but it was

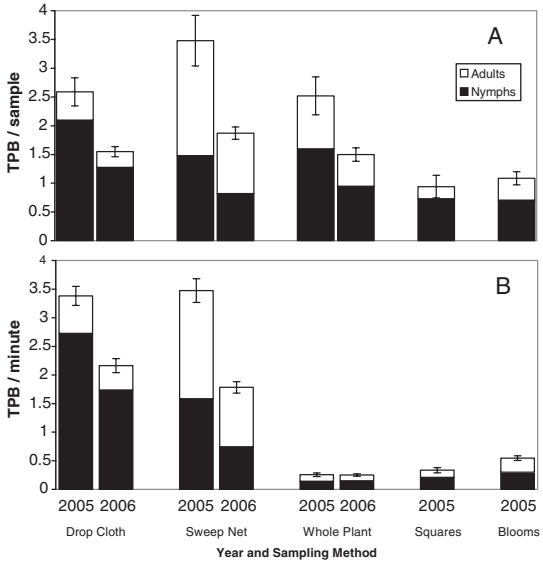

Fig. 1. Mean *L. lineolaris* adults and nymphs collected (A) per sample unit and (B) per minute of sampling in 2005 and 2006. Standard error bars are for total *L. lineolaris* collected.

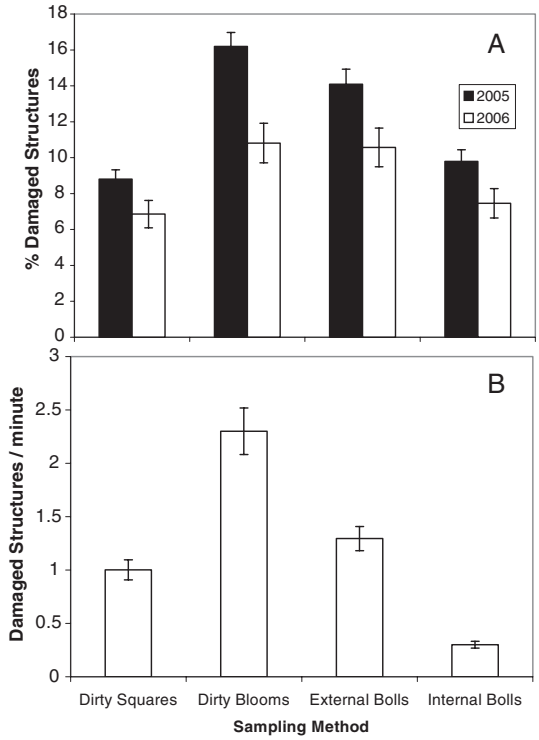

Fig. 2. Mean ± SEM bug damage observed (A) per sample unit in 2005 and 2006 and (B) per minute of sampling in 2006.

done to evaluate the frequency that a method missed or over-estimated an infestation.

Influence of External Factors. To evaluate the significance of uncontrolled factors influencing each of the methods, the sample mean was subtracted from each observation. PROC GLM (SAS Institute 1999) was then used on the difference to estimate the impact of uncontrolled factors on each sampling method. Factors considered were air temperature, cloud cover percentage, leaf wetness (dew present or absent), wind speed, plant height, and plant maturity measured as NAWF (Harris et al. 1997). Because there was substantial correlation among some of the factors (data not shown), each factor was individually evaluated. Discrete and quantitative factors were coded and evaluated as discrete classes. To detect linear associations with more power, quantitative factors also were evaluated directly. Where both analyses had a significant result, only the greatest significance is reported.

Sampling Efficiencies. Efficiency was estimated using the time required to collect a sample and the mean number of insects or damage recorded. However, all sampling methods do not share the same level of precision. To evaluate the sampling methods using efficiency and precision criteria together, we ranked fields from low bug or damage density to high bug or damage density for each sampling method. Fields with similar means were grouped together so that each grouping had ≈40–50 observations (e.g., four fields with 12 observations per field) as recommended by Binns et al. (2000) for estimating the relationship between mean and variance. This grouping produced at least eight independent point estimates of the mean and variance. The mean–variance relationship was then estimated for each method over the range of sampled densities using Taylor's power law, where variance = a × mean^b. These "a" and "b" coefficients, estimated using the worksheets developed by Binns et al. 2000) (http://www.nysaes.cornell.edu/ent/faculty/nyrop/cpdm), were then used with the converging lines simulation model to estimate the average number of samples required to make the right classification 80 and 90% of the time when the true pest density is 20% above the economic threshold. This simulation model was run 1,000 times for each sampling method. Minimum and maximum sample limits were adjusted as needed to reach the desired classification accuracy. These average sample numbers were then combined with the time required to collect a sample to estimate the sampling time required for that level of accuracy, thereby incorporating precision into the measure of efficiency. Sampling times from 2006 data were used for plant-based samples in both years because 2005 times were based on combinations of sampling methods rather than each sampling method alone.

Results

Tarnished plant bug represented 94% of the bug complex in both years. Due to the low numbers of other insects, the efficiency of direct sampling methods could only be analyzed for tarnished plant bug. Among the direct sampling methods, the sweep-net

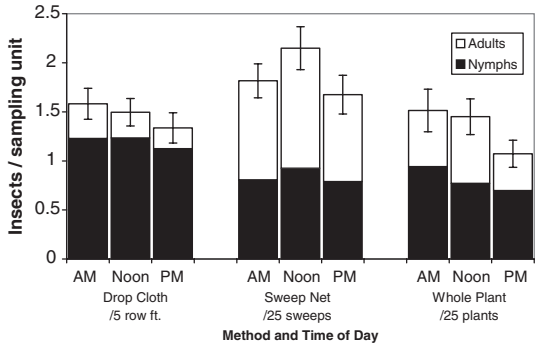

Fig. 3. Mean *L. lineolaris* adults and nymphs counted with direct sampling methods during the morning (6–9 a.m.), noon (11 a.m.–2 p.m.), and afternoon (4–7 p.m.) in 2006. Standard error bars are for total *L. lineolaris* collected.

method caught the most adults (2005: $F = 66.58$, df = 4,565, $P < 0.01$; 2006: $F = 23.71$, df = 2,252, $P < 0.01$), and the drop-cloth method caught the most nymphs per sample during both years (2005: $F = 33.72$, df = 4,565, $P < 0.01$; 2006: $F = 4.66$, df = 2,252, $P = 0.01$) (Fig. 1A). The sweep-net method caught more total *L. lineolaris* than drop-cloth or whole-plant methods per sample unit during 2005 ($F = 45.95$; df = 4,565; $P < 0.01$), but there were no significant differences among direct sampling methods in total *L. lineolaris* per sample during 2006 ($F = 1.76$; df = 2,252; $P = 0.17$). When the time required for collecting a sample unit was considered, more insects were collected per minute using the sweep net and drop cloth than with the other methods (Fig. 1B). Among indirect sampling methods, the most damage was observed using the dirty-blooms and external-boll methods (2005: $F = 15.57$; df = 3,447; $P < 0.01$; 2006: $F = 5.54$; df = 3,316; $P < 0.01$) (Fig. 2A). The dirty-blooms method was also the most rapid indirect sampling method so the most damage per minute was observed with the dirty-blooms method (Fig. 2B).

Total and nymph *L. lineolaris* counts were not significantly affected by time of day (total drop cloth $F = 1.00$; df = 2,162; $P = 0.37$; total sweep net: $F = 2.10$; df = 2,162; $P = 0.13$; total whole plant: $F = 2.40$; df = 2,162; $P = 0.09$; nymph drop cloth: $F = 0.51$; df = 2,162; $P = 0.60$; nymph sweep net: $F = 0.57$; df = 2,162; $P = 0.56$; nymph whole plant: $F = 0.14$; df = 2,162; $P = 0.87$) (Fig. 3). Time of day was not a significant factor for adult counts using the drop cloth ($F = 1.89$; df = 2,162; $P = 0.16$) and sweep net ($F = 1.31$; df = 2,162; $P = 0.27$) methods, but it was significant on whole-plant adult counts ($F = 7.91$; df = 2,162; $P < 0.01$) with counts being lowest during late afternoon when temperatures were hottest.

Table 3. Impact of white versus black drop cloths (both 76 × 91 cm) on counts of tarnished plant bug (TPB) in blooming cotton ($n = 60$)

Insect stage	Mean TPB per drop cloth		White to black	
	White	Black	Difference	% Change
Adult	1.17 ± 0.16a	0.98 ± 0.16a	−0.18 ± 0.19	−15
Nymph	7.43 ± 0.49a	9.55 ± 0.45b	2.12 ± 0.67	+29
Total	8.60 ± 0.50a	10.53 ± 0.46b	1.93 ± 0.69	+22

Means followed by the same letter in the same row are not significantly different at the 5% level (paired *t*-test).

The 2006 data were collected by 23 individuals in four states. Sampler variability was significant for all methods, with the dirty-square method having the least amount of variability (Table 2).

In the comparison of drop cloth fabric colors, 22% more *L. lineolaris* were observed on black drop cloths than on white drop cloths (Table 3). Counts of adults were not significantly different ($t = -0.97$, df = 59, $P = 0.34$), but more nymphs were counted on black drop cloths than on white drop cloths ($t = 3.18$, df = 59, $P < 0.01$), resulting in higher overall counts on black drop cloths than on white drop cloths ($t = 2.81$, df = 59, $P < 0.01$).

Correlations between individual sampling methods and the composite reference indicate that sweep-net, whole-plant, and dirty-bloom sampling methods had the strongest correlations to other methods in both years (Table 4). During 2005, recommendations generated by each sampling method based on equivalent pest densities also indicated that the sweep-net, whole-plant, and dirty-bloom methods had the strongest level of agreement with the other sampling methods (Table 4). However, during 2006 the highest levels of threshold agreement with other sampling methods were with the drop-cloth, whole-plant, and internal-boll methods. With the exception of square and bloom direct sampling methods that had small means, differences in precision among sampling methods were small.

The average number of samples required to reach a specified criterion (e.g., 80% correct decision when true density is 20% above threshold) was calculated

Table 2. Sampler impact on sampling method counts of tarnished plant bug (TPB) or bug damage

Sampling method	df	Total TPB or damage		TPB adults		TPB nymphs	
		F	*P*	*F*	*P*	*F*	*P*
Drop cloth	22, 486	3.08	<0.0001	4.00	<0.0001	2.87	<0.0001
Sweep net	22, 486	2.77	<0.0001	2.35	0.0006	3.16	<0.0001
Whole plant	22, 486	3.51	<0.0001	2.20	0.0014	4.67	<0.0001
Dirty wquares	22, 129	1.69	0.0380				
Dirty blooms	22, 132	2.26	0.0025				
External bolls	22, 128	5.63	<0.0001				
Internal bolls	22, 128	3.34	<0.0001				

Table 4. Coefficients of variation and correlation coefficients between individual sampling methods and a composite of all sampling methods

Sampling method	Correlation to composite (r)[a]		Coefficient of variation (%)		% Recommendations different from majority[b]	
	2005	2006	2005	2006	2005	2006
Direct						
Sweep net	0.859	0.811	75.5	94.3	20.5	16.7
Drop cloth	0.788	0.773	71.1	98.9	25.6	11.1
Whole plant	0.851	0.827	80.1	118.0	18.0	13.0
Squares	0.779		144.0		23.1	
Blooms	0.657		108.8		24.4	
Indirect						
Dirty squares	0.758	0.780	74.2	104.6	29.5	16.7
Dirty blooms	0.820	0.816	49.2	80.0	19.2	22.2
Internal bolls	0.758	0.711	83.2	95.0	29.5	14.8
External bolls	0.775	0.685	65.5	74.2	26.9	20.4

[a] All correlations are significant with $P < 0.0001$.

[b] Recommendation to control *L. lineolaris* in comparison with the recommendation of the majority of the nine and seven sampling methods in 2005 and 2006, respectively. The drop-cloth threshold was set at 1.6 bug equivalent per row-meter (Catchot 2005). Thresholds for other methods were based on the equivalent number of bugs captured over the two years. Thresholds used were sweep net, 12 *L. lineolaris* per 100 sweeps; whole plant, 9 *L. lineolaris* per 100 plants; squares, 3.5 *L. lineolaris* per 100 squares; blooms, 4 *L. lineolaris* per 100 blooms; dirty squares, 8% damaged; dirty blooms, 14% damaged; internal bolls, 9% damaged bolls; and external bolls, 12% damaged bolls.

using Taylor's power law (TPL) coefficients for each sampling method (Table 5). Most sampling methods required five to eight samples to meet the 80% correct criterion. The dirty-bloom method required fewer samples during 2005 but not during 2006. Counts of *L. lineolaris* in squares and blooms required many more samples than the other methods. The TPL coefficients varied among the 2 yr, changing the number of samples required to obtain 80% confidence in the recommendation. However, the average minutes required to accurately classify the density of a field were fairly consistent, demonstrating that the drop-cloth, sweep-net, and dirty-bloom methods were more efficient than the other methods evaluated.

Of the uncontrolled factors recorded, the factors listed in Table 6 were the only factors significantly correlated to the counts of a sampling method. Only two factor-method combinations were found to be significant during both years of research. The dirty-bloom method recorded more damage relative to other methods late in the season when plants were tall and squares and blooms were becoming less abundant, and the drop-cloth method detected relatively fewer insects late in the season.

Discussion

Overall data quality from sweep-net and drop-cloth sampling methods were similar, but they had different strengths and weaknesses. Although sweep nets caught more adults and had a stronger correlation with other sampling methods than drop cloths, more nymphs were detected on drop cloths. Neither method was significantly affected by time of day. Whole-plant sampling was effective but inefficient and affected by time of day. It is unlikely that pest

Table 5. TPL coefficients, average sample number, and time required to classify a field as above threshold 80 and 90% of the time when actual pest density is 20% greater than the threshold

Sampling method	Threshold	TPL coefficients		Avg sample no.		Minutes to classify	
		a	b	80% correct	90% correct	80% correct	90% correct
2005							
Sweep net	12/100 sweeps	0.685	1.462	4.7	10.7	5.1	11.7
Drop cloth	1.6/row-m	0.876	1.296	6.1	12.7	5.1	10.5
Whole plant	9/100 plants	0.936	1.268	6.2	12.8	66.5	137.2
Squares	3.5/100 plants	1.266	1.352	18.0	34.3	58.9	112.2
Blooms	4/100 plants	0.928	1.075	12.4	25.2	26.9	54.7
Dirty squares	8/100 plants	0.861	1.302	6.4	15.0	11.2	26.3
Dirty blooms	14/100 plants	0.841	1.072	3.5	8.9	4.4	11.3
External Bolls	12/100 plants	1.018	1.091	5.0	10.4	10.7	22.2
Internal bolls	9/100 plants	0.979	1.218	6.3	15.0	41.8	99.6
2006							
Sweep net	12/100 sweeps	1.427	1.220	7.4	13.1	7.5	13.4
Drop cloth	1.6/row-m	1.450	1.107	7.5	15.5	4.9	10.1
Whole plant	9/100 plants	1.572	1.316	8.6	26.0	47.0	142.2
Dirty squares	8/100 plants	0.983	1.192	6.9	13.2	12.1	23.1
Dirty blooms	14/100 plants	1.039	1.268	6.6	11.7	8.4	14.9
External bolls	12/100 plants	0.822	1.374	6.5	11.7	13.8	24.9
Internal bolls	9/100 plants	0.916	1.123	5.8	13.2	38.8	87.6

Table 6. Significant uncontrolled factors for counts after accounting for overall plant bug density from all methods in 2005 and 2006

Factor	Method	Yr	*F*	df	*P*	Interpretation
Date	Dirty squares	2005	4.03	3,71	0.011	More in late season
Date	Drop cloth	2006	5.79	3,157	0.001	More in late Aug.
Date	Sweep net	2006	3.90	1,159	0.05	More toward end of season
Date	Whole plants	2006	9.57	3,157	<0.001	More in late Aug.
Height	Dirty blooms	2005	12.62	1,73	0.001	More in taller plants
Height	Dirty blooms	2006	3.19	5,46	0.015	More on short and tallest plants
Height	Drop cloth	2006	8.06	1,156	0.005	More on taller plants
Height	External bolls	2006	10.93	1,50	0.002	More on shorter plants
Height	Sweep net	2006	11.70	1,156	0.001	More on taller plants
Height	Whole plants	2006	5.99	1,156	0.016	More on taller plants
NAWF	Drop cloth	2005	4.75	1,73	0.033	More when more NAWF
NAWF	Drop cloth	2006	3.43	5,155	0.006	More when two to five NAWF
NAWF	External boll	2006	3.15	5,47	0.016	More when few squares
NAWF	Sweep net	2006	4.44	5,155	0.001	Less when few squares
Temp.	Dirty blooms	2005	4.06	2,72	0.021	More at higher temperatures
Temp.	Drop cloth	2006	5.14	3,157	0.002	More at 30–32°C
Temp.	Whole plants	2006	3.84	3,157	0.011	Less at 30–32°C
Wetness	Sweep net	2005	4.63	1,73	0.035	More when leaves dry
Wind	Drop cloth	2005	13.59	1,73	<0.001	More at higher wind speed
Wind	Sweep net	2005	24.13	1,73	<0.001	More at higher wind speed
Wind	Whole plants	2006	6.56	1,159	0.011	More at lower wind speed

managers would routinely count *L. lineolaris* on enough plants (five to eight samples of 25 plants each) to make an accurate assessment, because this would take up to 1 hr per field compared with 5 min (plus walking time) with a sweep net or drop cloth. Dirty blooms were the most efficient indirect sampling method tested and generally produced a recommendation consistent with the other sampling methods. However, there is some concern that dirty blooms may be reflecting damage that is >1 wk old and therefore would not be effective soon after an insecticide application. The dirty-squares method displayed more recent damage, was relatively efficient, and showed less sampler variability than all other sampling methods. Both external and internal boll damage sampling methods were less efficient than most other sampling methods, had substantial sampler variability and likely also showed old damage.

Where a method recommendation differed from the majority recommendation, all methods had at least 20% incidence of both type I (method above threshold when majority below threshold) and type II (method below threshold when majority above threshold) errors (data not shown). Therefore, it seems that the use of any sampling method will result in approximately the same number of insecticide applications over the course of a growing season.

There were only two cases where an uncontrolled factor was associated with a change in sampling counts both years. Because *L. lineolaris* prefer cotton squares to other parts of the cotton plant, relatively more damage was recorded by the dirty-bloom method on the tallest and shortest plants when few squares were available. If this method is adopted for threshold determination, the number of squares available may need to be considered in setting the threshold. Fewer insects were found on drop cloths when cotton approached maturity. Tarnished plant bugs tend to be higher on the plant at this time where squares and small bolls are still available (Snodgrass 1998), so a lower proportion of the total population is likely to land on the drop cloth. The other uncontrolled factors measured were not consistently associated with increased or decreased counts by any sampling method. Therefore, it is thought that within the typical ranges encountered for these factors, thresholds do not need to be adjusted for these factors.

Our drop cloth to sweep net comparisons are consistent with others showing that sweep nets collect more adults but drop cloths collect more nymphs (Young and Tugwell 1975, Fleischer et al. 1985, Snodgrass 1993, Stewart et al. 2001). Young and Tugwell (1975) also found various visual search methods to be much less efficient than the sweep net or drop cloth methods. Gore (2005) found plant-based methods in general and specifically the dirty-square method gave the highest correlations to yield. In this study, yield was not monitored, but the dirty-square and dirty-bloom methods were comparable with the best direct methods in accuracy and efficiency, so they merit further examination.

Based on these comparisons of sampling methods, further research is being conducted to develop thresholds using black drop cloths, sweep nets, dirty blooms, and dirty squares as these methods seem to be the most promising sampling methods currently available for monitoring tarnished plant bugs in cotton. Further research also is needed to develop a complete sampling protocol for the most promising methods. Because there were relatively strong correlations among these sampling methods (data not shown), it is expected that any of these sampling methods could be reliably used to make management decisions in most situations and that the choice of sampling method will primarily depend on the preferences of the sampler.

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